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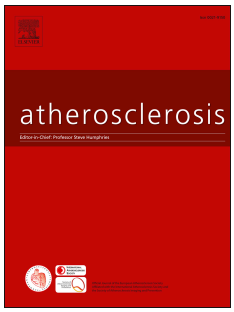

The association between plasma matrix metalloproteinase-9 concentration and endoleak after endovascular aortic aneurysm repair: A meta-analysis

Eugene Ng, Dylan R. Morris, Jonathan Golledge

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TITLE PAGE

Title

The association between plasma matrix metalloproteinase-9 concentration and endoleak after endovascular aortic aneurysm repair: A meta-analysis

Authors

Eugene Ng[1,2,3], Dylan R. Morris[2], Jonathan Golledge[1,2,3]

[1]Department of Vascular and Endovascular Surgery, Townsville Hospital, Queensland, Australia

[2]Queensland Research Centre for Peripheral Vascular Disease, College of Medicine and Dentistry, James Cook University, Townsville, Australia

[3]School of Medicine, University of Queensland, St Lucia, Australia

Corresponding Author

Professor Jonathan Golledge

Queensland Research Centre for Peripheral Vascular Disease

College of Medicine and Dentistry

James Cook University

Townsville, QLD Australia

jonathan.golledge@jcu.edu.au

Phone: +61 7 4781 4730

Fax: +61 7 4781 3652

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ABSTRACT

Background

The most common complication after endovascular aneurysm repair (EVAR) is continued perfusion of the aneurysmal sac, known as endoleak. Assessment of markers released from the aneurysm wall into the circulation has been suggested as a possible alternative for detecting endoleaks. The aim of this meta-analysis was to examine if circulating concentrations of matrix metalloproteinase (MMP)-9 were higher in patients with endoleak after EVAR.

Methods

A systematic search of the MEDLINE, EMBASE, Scopus, Web of Science and Cochrane Library Databases was conducted. Studies reporting circulating MMP-9 concentrations in patients who did and did not have endoleaks after EVAR that met inclusion and exclusion criteria were included. A meta-analysis using a random effects model was performed to assess the association between circulating concentrations of MMP-9 and endoleak. Sensitivity analyses were performed using the one-study remove approach. Study quality was assessed using a quality assessment tool.

Results

Prior to EVAR, plasma concentrations of MMP-9 were similar in patients that did and did not subsequently develop an endoleak (Standardised mean difference: -0.13; 95% confidence interval, -0.63-0.37, p=0.60). 1 month after EVAR, plasma concentrations of MMP-9 were non-significantly higher in patients that had an endoleak (Standardized mean difference: 0.56; 95% CI -0.02-1.15, p = 0.06). 3 months after EVAR, plasma concentrations of MMP-9 were higher in patients that had an endoleak (Standardised mean difference: 1.42; 95% confidence interval, 0.48-2.36, p<0.003).

Conclusions

This meta-analysis suggests that plasma MMP-9 concentrations measured 3 months after EVAR are higher in patients that have an endoleak. It remains to be established whether plasma MMP-9 testing is sufficiently accurate for use as a surveillance test for endoleak after EVAR.

INTRODUCTION

The global mortality attributable to aortic aneurysm rupture is estimated as 2.78 per 100,000 person years, which represents an 11% increase over the last 20 years.[1] The only recognized treatment for aortic aneurysm is surgical repair which is predominantly performed using stent grafts placed inside the aorta, i.e. endovascular aortic aneurysm repair (EVAR). The most common complication of EVAR is residual perfusion of the aneurysmal sac known as endoleak. Endoleak can be defined into 5 main types. Residual blood flow into the aneurysm sac occurring proximal or distal to site of the graft attachment is known as Type 1. Reverse flow through aortic branches, such as visceral, lumbar or thoracic arteries, into the aneurysm sac are known as type 2 endoleaks and are the most common type. Type 3, 4 and 5 endoleaks are due to graft defects, graft porosity and endotension, respectively, and are rare. Due to the frequent occurrence of endoleaks patients undergo long term surveillance after EVAR with computed tomographic angiography (CTA) and ultrasound imaging. A number of potential problems are associated with the use of these imaging modalities. CTA necessitates contrast administration and radiation exposure, posing a problem in patients with impaired renal function and also exposing the patients to a small increased risk of malignancy. Ultrasound, while eliminating the problem of contrast and radiation exposure, requires experienced sonographers and is accepted to not be as accurate as CTA. The use of both these imaging modalities require trained staff and appropriately equipped facilities which may pose a problem to patients based remotely or regionally. The use of these modalities for surveillance also reduces the cost effectiveness of EVAR.

A number of circulating biomarkers have been reported to be associated with the presence of aortic aneurysm, such as markers of thrombosis, inflammation and matrix degradation.[2] Matrix metalloproteinases (MMPs) are soluble, zinc-dependent enzymes with elastolytic activity and are produced by a range of cells including macrophages and smooth muscle cells. MMPs, particularly MMP-9, have been implicated in the development of aneurysms through extracellular matrix degradation leading to structural changes in the aortic wall which stimulates excessive positive remodeling.[3] Release of MMP-9 into the circulation has been suggested as a biomarker for abdominal aortic aneurysm (AAA) presence, progression and complications of AAA repair. Continued perfusion of the aneurysm sac in individuals with endoleak is postulated to promote MMP-9 release into the circulation.[4]

The objective of this meta-analysis was to evaluate whether circulating concentrations of matrix metalloproteinase (MMP)-9 were higher in patients who developed endoleak compared to patients who did not have an endoleak after EVAR.

METHODS

Literature Search

A search strategy was devised according to the 2009 Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA) statement.[5] An electronic search of the databases MEDLINE, EMBASE, SCOPUS, Web of Science and Cochrane Library Databases was performed from inception to December 2014 with no language restrictions on 25th November 2014. To identify studies investigating plasma levels of MMPs and the presence of endoleak after EVAR, the following search terms were applied: ('Endovascular repair of aortic aneurysm' OR 'EVAR')[Title/Abstract] AND ('endoleak')[Title/Abstract] AND ('biomarker*' OR 'MMP' OR 'matrix metalloproteinase')[Title/Abstract]. The database search was supplemented by a search of the reference lists of included studies as well as utilizing the related articles function provided in each database. Titles and abstracts were screened to identify potentially relevant studies. If the suitability of an article was uncertain, the full text article was reviewed. All potentially relevant studies were subsequently assessed by review of the full text articles. Eligible studies were those that assessed MMP-9 levels and endoleak in patients who had undergone EVAR of aortic aneurysms. Included studies utilized CTA in the identification of endoleak. Studies were excluded if animal studies were involved; there was no clear division of patients into endoleak and non-endoleak groups; endovascular repair of aneurysms involving arteries other than the aorta was performed; and if the assessment of circulating blood markers did not include MMP-9. Review articles were also excluded.

Data extraction

Data extraction was performed according to a predefined form and recorded in tables. All data was reviewed independently by 2 authors (E.N and D.R.M) and cross-checked in a consensus meeting. Any discrepancies were resolved through discussion. The following data were obtained from the included studies: Study author, year and design, study sample size, individual study inclusion and exclusion criteria, population characteristics, definitions used for endoleak, type of aneurysm repaired, number and type of stent grafts used, number of endoleaks, MMP-9 assay used, mean concentration and variance of circulating plasma MMP-9 concentrations in patients with and without endoleak, imaging modality for detection of endoleak, the timing of imaging after EVAR and the timing of blood collection for MMP-9 measurements after EVAR. If blood samples were collected at multiple time points, then all measured MMP-9 concentrations were recorded. Authors of eligible studies were contacted for additional information when required.

Quality Assessment

The quality and potential bias of included studies were assessed by 2 of the authors (E.N, D.R.M). A modified quality assessment form was derived using elements from the Newcastle

Ottawa Scale and Cochrane collaboration tool for assessment of the risk of bias.[6-7] This form is summarised in Supplementary Table 1 and a discussion is provided in the Appendix.

Statistical Analysis

A meta-analysis was performed comparing plasma MMP-9 concentrations in blood samples obtained preoperatively, 1 month after EVAR and 3 months after EVAR in patients with and without an endoleak identified during follow-up. These time points were chosen as they were each reported in three or more eligible studies. Three studies[8,9,10] reported MMP-9 levels as means and standardized deviation (s.d.) and two studies[11,12] reported means and standard error. All data was imported into Review Manager (Revman, Version 5.2, Copenhagen; The Nordic Cochrane Centre, Copenhagen, Denmark). Standard deviations were calculated automatically by the Revman software for studies that reported standard error only. Standardised mean differences (SMDs) and 95% confidence intervals (CI) were calculated for each included study. Study specific estimates were combined using inverse-variance weighted average of logarithmic SMDs in a random-effects model. The random-effects model was chosen to assess heterogeneity on the summary statistics. Inter-study heterogeneity was assessed by means of the I^2 index. I^2 values of >50% were accepted to denote statistical heterogeneity. Sensitivity analyses were performed using the one-study-removal approach to assess the contribution of each study to the pooled estimate[6] (Supplementary table 4). This was achieved by excluding individual studies one at a time and recalculating the pooled SMD estimates for the remaining studies. Publication bias was assessed by constructing funnel plots of the logarithm of effect size versus the standard error, however, accurate appraisal was not possible due to the limited number of studies.

RESULTS

Study selection

Initial database searches yielded fifteen potentially eligible studies after removal of duplicates (Figure 1). No additional studies were identified by hand-searching the reference lists. A total of seven studies were excluded based on review of their titles and abstracts. The most common reasons for exclusion were failure to measure MMP-9 concentrations or identify endoleaks in patients who had undergone EVAR.

Of the eight studies[8-15] reporting plasma MMP-9 concentrations in patients who had been investigated for endoleak after EVAR, three studies were excluded based on analysis of their full text.[13,14,15] One study was a review article which was excluded as defined in our exclusion criteria.[13] One study reported no identified endoleaks even though MMP-9 concentrations were measured.[14] One study reported endoleaks but did not report values for MMP-9 concentrations and data could not be obtained from contacting the authors.[15]

Study characteristics

Ultimately five studies[8-12] were included in this meta-analysis (Figure 1). Of the included studies, two studies were conducted in the United States[10,11], one study in Italy[8], one in the Netherlands[9] and one in Japan[12]. All studies were hospital based and consisted of either vascular surgical inpatients or individuals presenting to a vascular clinic for follow-up. The design of the included studies are outlined in Table 1 and a summary of each individual study findings is provided in Table 2. Four of the studies included were prospective cohort studies[8,9,10,11] and one study was a non-randomized control trial[12]. Four studies[9-12] involved AAA patients while 1 study[8] involved thoracoabdominal aneurysm patients. The average age of the studied patients ranged from 66 to 76 years. There were no significant differences in the sex of patients that had and did not have an endoleak in all studies. All studies[8-12] provided baseline characteristics of patients including at least age, gender and vascular comorbidities. Sample sizes of the included studies ranged from 4 to 17 patients with an endoleak and 13 to 23 patients with no endoleak. Overall the studies selected in this meta-analysis included a total of 127 patients, of whom 40 had endoleaks identified on CT imaging. The types of endoleaks included type 1 (n=12), type 2 (n=27) and type 3 (n=1). In one study[11], although there were 8 endoleaks, identified samples were only available for 5 patients for assessment of MMP-9 levels. Therefore, a total of 37 endoleaks were included in the meta-analysis.

Seven different types of stent grafts were used in the 4 studies[8,10,11,12] that provided data including Ancure Guidant (16), Gore Excluder (25), Talent World Medical (39), AneuRx Medtronic (6), Vanguard (7), Cook Zenith (13) and custom made Malmo-Ivancev, consisting of a non-crimped Dacron graft handsewn to a tapered Gianturco stent with Goretex sutures (3). One study did not state the type of stent grafts used[9].

All studies[8-12] measured plasma MMP-9 levels as a primary outcome measure. In addition to MMP-9 levels, two studies[8,10] measured MMP-3 concentrations, two studies[8,9] measured tissue inhibitor of metalloproteinase (TIMP-1) and one study [9] measured MMP-2 levels. Given the limited number of studies investigating these two circulating markers, a meta-analysis was only conducted for MMP-9.

All studies[8-12] used different commercially available ELISA assays to estimate plasma MMP-9. Three studies[8,10,11] used an assay from Amersham UK; one study[12] used an assay from SRL Inc, Japan and one study [9] used an assay from GE Healthcare, Sweden. Four of the studies[8,10,11,12] reported the protocol for MMP-9 measurement. Two studies[10,11] reported the within assay (<6%) and between assay precision (<10%) while the remaining three studies[8,9,12] did not report assay variability.

Plasma MMP-9 levels were assessed at a variety of time points. All five studies[8-12] reported MMP-9 values at 3 months after EVAR (Table 3). One study[11] also reported the trend in MMP-9 levels in patients with and without endoleak. Two studies[11,12] measured plasma MMP-9 levels

prior to EVAR, 1 month and 3 months after EVAR. Two studies[8,10] measured plasma MMP-9 levels prior to EVAR, 1 month, 3 months and 6 months after EVAR. One study[9] did not define the time point for measurement of MMP-9.

All studies[8-12] utilized CTA in the diagnosis of endoleak. The time points used for CTA surveillance imaging for endoleak was variable across studies. One study[11] performed CTA at the time of discharge, 1 and 3 months after EVAR. One study[10] performed CTA at the onset of new symptoms and at 6 months after EVAR. One study[8] performed CTA on discharge, 1 and 6 months after EVAR. One study[12] performed CTA at 3 and 12 months after EVAR. One study[9] did not report when CTA was performed.

Mean follow up time was variable across studies, ranging from 6 to 25 months. Only two studies [8,10] reported clear follow up time periods. The remaining three studies[9,11,12] did not report the follow up times even though plasma MMP-9 measurements and surveillance CTA were conducted at variable time points after EVAR.

All studies[8-12] reported significantly higher MMP-9 levels in patients with endoleak when compared to patients without endoleak at various timepoints. Two studies[8,12] reported higher plasma MMP-9 levels in patients that had an endoleak compared to patients that had no endoleak at 1 month after EVAR. Three studies[8,11,12] reported higher plasma MMP-9 levels in patients that had an endoleak compared to patients with no endoleak at 3 months after EVAR. One study[10] reported higher plasma MMP-9 levels in patients that had an endoleak at 6 months after EVAR. One study[8] reported higher plasma MMP-9 levels in patients that had an endoleak at 1 month, 3 months and 6 months after EVAR. One study[9] did not state when plasma MMP-9 levels were measured but noted higher plasma MMP-9 levels in patients who had endoleaks when compared to patients without endoleaks.

One study[9] assessed the sensitivity and specificity of plasma MMP-9 in detecting endoleak presence by use of a receiver operating characteristic curve and reported sensitivity of 100% and specificity of 96% at a cutoff MMP-9 value of 55.18ng/ml.

Study Quality

None of the studies were graded as good according to predefined quality criteria. Three studies[8,10,11] were graded as average while the other two remaining studies[9,12] were graded as poor (Table 3 and Appendix).

Data Synthesis

Meta-analysis was performed to analyze MMP-9 concentrations prior to EVAR (4 studies[8,10,11,12]), 1 month after EVAR (3 studies[8,10,12]) and 3 months after EVAR (5 studies[8-12]) according to the availability of data.

A meta-analysis of four studies[8,10,11,12] consisting of 90 patients, 20 of whom developed an endoleak, found no significant differences in pre-operative plasma MMP-9 concentrations between patients who did and did not subsequently develop an endoleak (Figure 2; SMD: -0.14; 95% CI -0.64-0.36, p=0.60). No significant heterogeneity was noted in these four studies (I^2=0).

A meta-analysis of three studies[8,10,12] consisting of 72 patients, 15 of whom developed an endoleak showed a trend towards higher MMP-9 levels in patients with endoleak but this did not reach statistical significance (Figure 3; SMD: 0.56; 95% CI -0.02-1.15, p = 0.06). No significant heterogeneity was noted in these 3 studies (I^2=0).

A meta-analysis of all five studies[8-12], consisting of 37 patients with endoleaks and 90 patients without endoleaks, demonstrated significantly higher plasma concentrations of MMP-9 in patients who developed endoleaks compared to those who did not 3 months after EVAR (Figure 4; SMD: 1.42; 95% CI 0.48-2.36, p<0.003). There was evidence of heterogeneity across these studies (I^2=75%) and the likelihood of publication bias could not be assessed due to the limited number of studies. Sensitivity analyses through the one-study-remove approach demonstrated that multiple studies contributed to the observed difference in plasma MMP-9 levels between the two groups and that exclusion of any one study did not substantively effect the overall result.

DISCUSSION

The number of studies which have investigated biomarkers concentrations prior to and after EVAR are limited. Our meta-analysis suggests that plasma MMP-9 levels are higher in patients that have endoleaks 3 months after EVAR compared to patients that have successful exclusion of their aneurysm. The meta-analysis also suggested that plasma MMP-9 concentrations were higher in patients with endoleaks 1 month after EVAR, however, the finding was of borderline significance (p=0.06), most likely due to the reduced sample size for this time point. It is postulated that continued perfusion of the aneurysmal sac in patients that have an endoleak perpetuates the aortic wall remodeling resulting in release of MMP-9 into the circulation.

In contrast, our meta-analysis suggested that pre-operative plasma MMP-9 levels were not significantly different in patients who did and did not subsequently develop an endoleak. In four of the included studies[8,10,11,12], it was reported that plasma MMP-9 levels declined after EVAR in patients with successful exclusion of their aneurysm. The time point after EVAR when blood samples were collected for MMP-9 measurement varied in the included studies. Pre-operative MMP-9 levels were compared with those measured in samples obtained between 1 and 6 months after EVAR.[8-12] One study[9] did not report baseline MMP-9 levels but reported significantly higher plasma MMP-9 levels in patients who had endoleak when compared to patients with no endoleak after EVAR.

At present CTA is the gold standard for detecting endoleaks after EVAR. CTA necessitates contrast administration and radiation exposure, posing a problem in patients with impaired renal function and also exposing the patients to a small increased risk of malignancy. Alternative forms of imaging used include ultrasound, contrast enhanced duplex ultrasound, MRI and nuclear imaging.[16] The use of CTA as an imaging modality requires trained staff and appropriately equipped facilities which reduces the cost effectiveness of EVAR.

An ideal biomarker for use in the detection of endoleaks would have high sensitivity to detect all true endoleaks as well as a high specificity to exclude individuals with no endoleak. The sensitivity and specificity of MMP-9 as a biomarker to detect endoleak was investigated in one of the included studies.[9] The authors utilized a receiver operating characteristic curve to assess the sensitivity and specificity of plasma MMP-9 in detecting endoleaks. The study[9] reported a sensitivity of 100% and a specificity of 96% at a cutoff MMP-9 value of 55.18ng/ml. This study[9] included only 37 patients and larger prospective studies are required to confirm the findings. It is possible that the use of MMP-9 might reduce the requirement for imaging after EVAR by limiting CTA to patients with concentrations above this or similar levels however this needs specifically investigating.

The majority of endoleaks detected in this study were Type 2. Previously defined risk factors for endoleak include large aneurysm diameter, severe infrarenal neck angulation, short infrarenal neck length, the presence of concomitant iliac artery aneurysm, female sex, history of smoking, and hypertension.[17-19] It has not been investigated whether plasma MMP-9 can differentiate between the various subtypes and degrees of endoleak. A biomarker for endoleak that is both qualitative and quantitative would be extremely useful especially with regards to directing subsequent intervention. Future studies could investigate the association between MMP-9 levels and type of endoleak.

This meta-analysis has a number of limitations. Overall study quality ranged from poor to average with significant heterogeneity between studies. There was variation in the time intervals used for assessment of endoleak and measurement of plasma MMP-9. Our finding of a higher plasma MMP-9 level in patients with endoleak was based on data reported at 3 months after EVAR. A uniform surveillance protocol would be useful in future studies to examine whether endoleaks detected at later follow-up stages are also associated with higher MMP-9 concentrations. In additional, current practice for follow-up of EVAR now extends up to 10 years. The maximal follow-up period in our meta-analysis post EVAR was 3 months and this limits conclusions that can be drawn due to the short length of follow-up.

None of the included studies adjusted for potential confounding factors. It is possible that elevated MMP-9 levels in patients with endoleak could reflect another feature of these patients other than endoleak. It has been reported for example that greater systemic atherosclerosis increases the risk of endoleak and atherosclerosis itself is associated with higher circulating MMP-9 levels. Furthermore, one study[12] involved administration of azelnidipine (a calcium

channel blocker) which may possibly contribute to a decrease in plasma inflammatory biomarkers including MMP-9. None of the studies adjusted for statin use, which has been associated with attenuation of MMP-9 activity in aortic aneurysmal tissue.[20,21]

There was variability in the measurement of MMP-9 concentrations across studies. Three studies[8,10,11] utilised the same assay from the UK while two other studies utilized assays from Sweden[9] and Japan[12]. Furthermore, MMP-9 exists as a secreted proenzymatic zymogen which is then cleaved into its active 82kDa form by peptidases and other MMPs. Both forms can be found in plasma. None of the studies in this meta-analysis defined exactly which form was measured. Therefore, it is impossible to ascertain which MMP-9 form was associated with endoleak after EVAR.

Finally, there was a relatively small number of patients in all the included studies with a pooled total of only 127 patients. This limits the conclusions that can be drawn as a result. Yet, it is remarkable that MMP-9 levels were significantly elevated in all the included studies[8-12], despite the small sample numbers. Larger clinical studies in which blood samples are collected at standardized time points and patients are prospectively included are needed to verify the findings of this meta-analysis. Studies are also needed to assess the association of other biomarkers with endoleak.

CONCLUSIONS

Studies investigating the relationship of plasma MMP-9 and endoleak after EVAR are limited to small poor quality studies. Even though this meta-analysis suggests that plasma MMP-9 concentrations are higher in patients with endoleak 3 months after EVAR, further studies encompassing larger patient numbers with stringent reproducible protocols are needed to confirm these findings.

DISCLOSURES

The authors have no disclosures.

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Figure Legends

Figure 1: PRISMA flow diagram to illustrate selection of studies

Figure 2. Meta-analysis of the association between plasma MMP-9 levels measured prior to EVAR and the development of endoleak. Forest plot illustrating mean MMP-9 concentrations with 95% CIs. The diamond represents the overall mean MMP-9 concentration calculated with a random effects model.

Figure 3. Meta-analysis of the association between plasma MMP-9 levels and presence of endoleak measured 1 month after EVAR. Forest plot illustrating mean MMP-9 concentrations with 95% CIs. The diamond represents the overall mean MMP-9 concentration calculated with a random effects model. Higher levels of MMP-9 were demonstrated in patients with endoleak.

Figure 4. Meta-analysis of the association between plasma MMP-9 levels and presence of endoleak measured 3 months after EVAR. Forest plot illustrating mean MMP-9 concentrations with 95% CIs. The diamond represents the overall mean MMP-9 concentration calculated with a random effects model. Higher levels of MMP-9 were demonstrated in patients with endoleak.

Table 1: Characteristics of the included studies

Study	Type of study	Inclusion criteria	Exclusion criteria	Number of EVARs	Number of endoleaks	Aneurysm type	Imaging surveillance protocol	EVAR stent graft type	Biomarkers measured	Time between EVAR and MMP measurement
Monaco et al, 2007[8]	Prospective cohort	Atherosclerotic TAA Aneurysmal diameter >6cm Documented aneurysmal growth more than 0.5cm in last 12 months	Thoracic aortic dissection Thoracic aortic transection Marfan Syndrome Proximal aortic neck diameter >4.4cm Length of nonaneurysmal aorta from origin of left subclavian artery to proximal attachment site <1.5cm	32	4	TAA	CTA on discharge, 1 month and 6 months postoperatively	Talent (Medtronic)	MMP-9 MMP-3 TIMP-1	Baseline 1 month 3 months 6 months
Hellenthal et al, 2012[9]	Prospective cohort	Not stated	Not stated	37	17	AAA	CTA (period not stated)	-	MMP-9 MMP-2 TIMP-1	Baseline 18 months 21 months
Sangiorgi et al, 2001[10]	Prospective cohort	Aneurysmal diameter >4cm Documented aneurysmal expansion >5mm in 12 months	Aortic neck diameter <20mm <1.5cm of nonaneurysmal infrarenal aorta at proximal attachment site Angulation of aorta >60 degrees Aberrant renal arteries Tortuosity or kinking of iliac vessels	30	7	AAA	CTA, 6 months postoperativelyand on onset of new symptoms	Gore Excluder (n=16) Vanguard (n = 7) Talent World Medical (n = 7)	MMP-9 MMP-3	Baseline 1 month 3 months 6 months
Lorelli et al, 2002[11]	Prospective cohort	All elective AAA	Aneurysmal rupture Unwilling participants	25	8	AAA	CTA, 1 month and 3 month postoperatively CTA, before discharge and at 3 months postoperatively (custom-made stent graft)	AneuRx Medtronic (n = 6) Ancure Guidant (n = 16) Custom made Malmo-Ivancev (n = 3)	MMP-9	Baseline 1 month 3 months
Nakamura et al, 2009[12]	Non-randomised RCT	Atherosclerotic infrarenal true AAA with diameter > 45mm Availability for followup	Clinical/laboratory suspicion of infection Acute aortic dissection Traumatic aortic lesions Ruptured aneurysms Mycotic aneurysms CRF, on dialysis	22	4	AAA	CTA (preop, 3 months and 1 year postoperatively)	Cook Zenith (7 in azelnidipine, 6 in control) Gore Excluder (5 in	MMP-9 AAA diameter	Baseline 1 month 3 months

			Autoimmune disease Connective tissue disease Aortitis Neoplasms Immunodeficiencies Anti-inflammatories Chemotherapy/Immunosuppresant Indication for open surgery Difficulty in postoperative followup					azelnidipine, 4 in control)		

RCT: randomized controlled trial EVAR: endovascular aneurysm repair AAA: abdominal aortic aneurysm CTA: computed tomographic angiography MMP-2: matrix metalloproteinase-2 MMP-3: matrix metalloproteinase-3 MMP-9: matrix metalloproteinase 9 TAA: thoracic abdominal aneurysm TIMP-1 tissue inhibitor of metalloproteinase-1 CRF: chronic renal failure EVAR: endovascular repair of aneurysm MMP-9: matrix metalloproteinase-9

Table 2: Summary of included studies

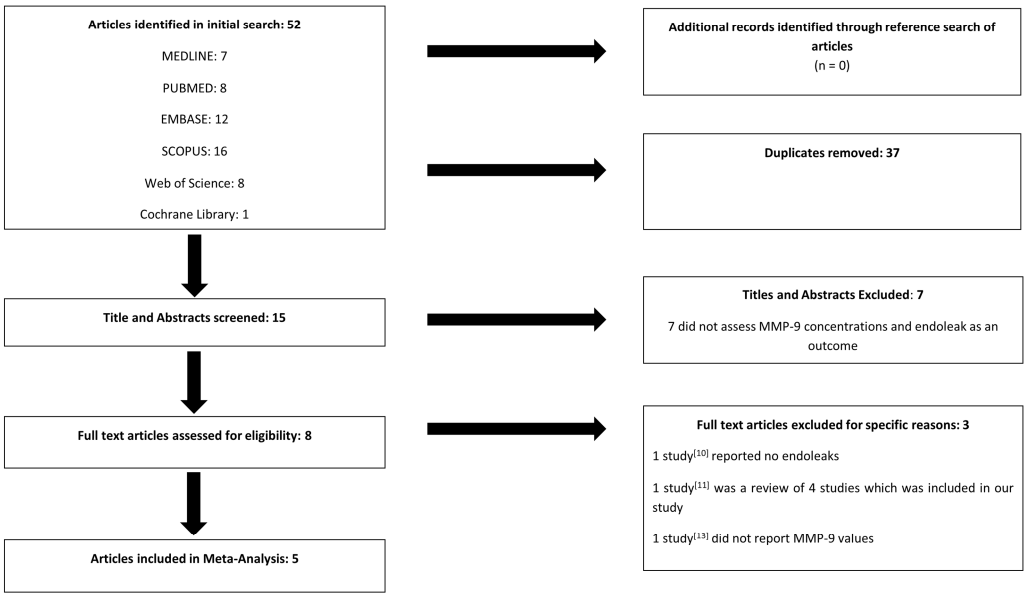
Study	Study Design	Selected Study Outcomes
Monaco et al, 2007[8]	Evaluation of plasma MMP-9, MMP-3 and TIMP-1 levels in patients undergoing EVAR (32 vs 25 control)	In patients who underwent EVAR without endoleak, plasma MMP-9 levels were not significantly reduced postoperatively, at 1 month, 3 months and 6 months (p=NS) when compared to control group values. In patients who underwent EVAR with endoleak, mean plasma MMP-9 levels were significantly higher postoperatively (p<0.01), 1 month (p<0.001), 3 months (p<0.01) but not significantly increased at 6 months (p=NS) when compared to control group values. In patients who underwent EVAR with endoleak, mean plasma MMP-9 levels were higher at 1 month (P<0.02), 3 months (p<0.02) and 6 months (p<0.04), when compared to patients who underwent EVAR without endoleak
Hellenthal et al, 2012[9]	Evaluation of plasma MMP-9 levels in patients undergoing EVAR	In patients who underwent EVAR with endoleak, mean plasma MMP-9 levels were significantly higher when compared to patients who underwent EVAR with no endoleak (89.54±26.46ng/ml vs 25.02±13.40ng/ml respectively, p<0.0001)
Sangiorgi et al, 2001[10]	Evaluation of plasma MMP-9 and MMP-3 levels in patients undergoing open surgery or EVAR (15 vs 30)	In patients who underwent EVAR, there were 7 cases of endoleak, 5 Type 1 and 2 Type 2. In patients who underwent open repair, mean plasma MMP-9 levels were significantly reduced from baseline (28.8+9.9ng/ml) at 6 months (14.7±6.6ng/ml, p<0.001) In patients who underwent EVAR without endoleak, mean plasma MMP-9 levels were significantly reduced from baseline (34.1±22.6ng/ml) at 6 months (14.6±7.0ng/ml, p<0.05)

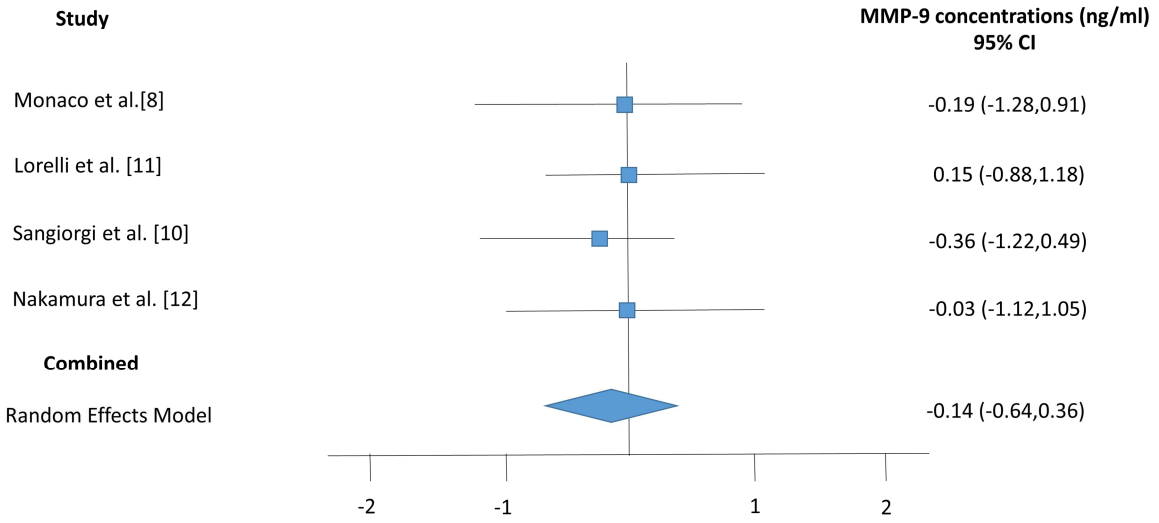
		In patients who underwent EVAR with endoleak, mean plasma MMP-9 levels were significantly increased from baseline (34.1±22.6ng/ml) at 6 months (44.3±20.7ng/ml, p<0.05)
Lorelli et al, 2002[11]	Evaluation of plasma MMP-9 levels in patients undergoing open surgery or EVAR (26 vs 25)	In patients who underwent open repair, mean plasma MMP-9 levels were significantly increased from baseline (83.9±26.1ng/ml) at 1 month (149.5±40.1ng/ml, p<0.05) and remained elevated at 3 months (129.8±56.6ng/ml) In patients who underwent EVAR without an endoleak, mean plasma MMP-9 levels were significantly reduced from baseline (60.8±8.8ng/ml) at 3 months (27.4+5.2ng/ml, p<0.05) In patients who underwent EVAR with endoleak, mean plasma MMP-9 levels were not significantly reduced from baseline (53.0±11.2ng/ml) at 3 months (67.1±26.7ng/ml, p= NS)
Nakamura et al, 2009[12]	Evaluation of plasma MMP-9 in patients undergoing EVAR randomized to receive azelnidipine and control (12 v 10)	In patients who underwent EVAR, there were 4 cases of endoleak (Type 2), 3 in azelnidipine, 1 in control In patients with no endoleaks after EVAR, mean plasma MMP-9 levels decreased significantly (from 39.5±14.3ng/ml to 25.0±12.6, p = 0.004) and 3 months (28.2±10.2ng/ml, p = 0.004) In patients with endoleaks, no significant reduction in mean plasma MMP-9 from baseline (37.5±9.0ng/ml) at 1 month (26.8±8.4ng/ml) and 3 months (38.5±15.7ng/ml) In the azelnidipine group without endoleaks, mean plasma MMP-9 decreased significantly from baseline (47.7±13.2ng/ml) after 1 month (26.6±12.8ng/ml, p<0.001) and 3 months (26.1±11.4ng/ml, p<0.001) In the control group without endoleaks, no significant reduction in mean plasma MMP-9 was noted from baseline (31.3±10.5ng/ml) at 1 month (33.4±12.1ng/ml) and 3 months (30.3±9.1ng/ml)

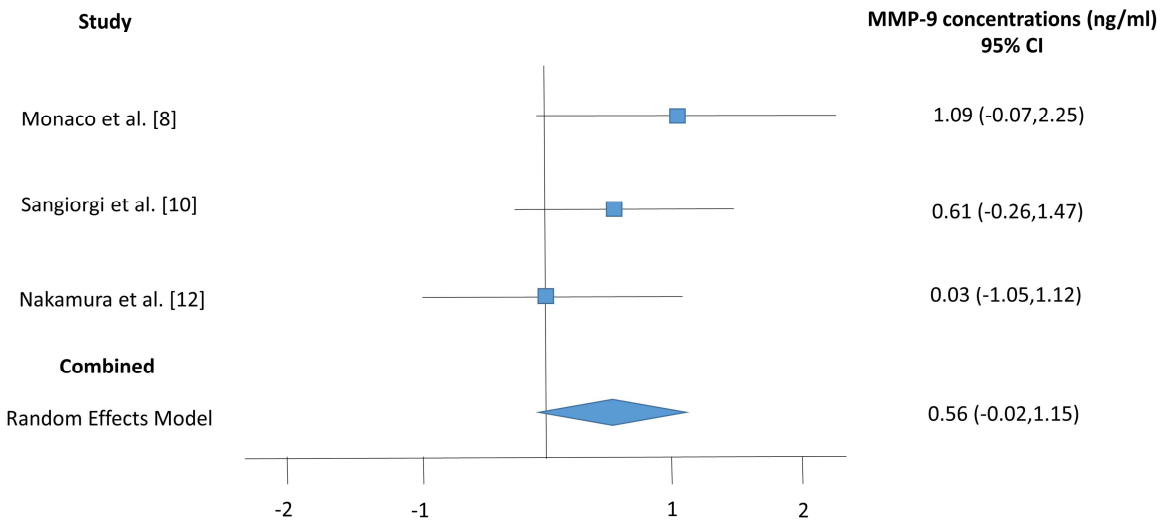
Table 3: Mean MMP-9 plasma levels (ng/ml) in patients with and without endoleak at 3 months after EVAR

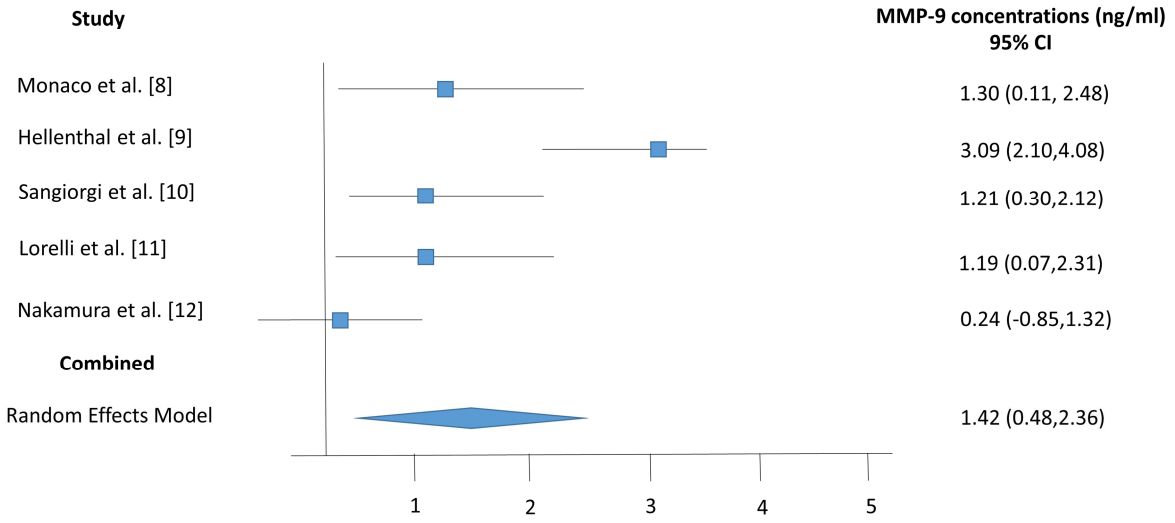
Study	Endoleak			No endoleak			Weighted mean (%)	Standardised Mean Difference	p value	Assay Brand
	Mean	SD	Total	Mean	SD	Total				
Monaco et al, 2007[8]	130.3	32.2	4	70.4	46.2	16	16	1.30 (0.11,2.48)	0.03	Amersham, UK
Hellenthal et al, 2012[9]	89.5	25.5	17	25.0	13.4	20	29	3.09 (2.10, 4.08)	0.00001	GE Healthcare, Sweden
Sangiorgi et al, 2001[10]	40.2	20.9	7	23.6	10.4	23	24	1.21 (0.30, 2.12)	0.009	Amersham, UK
Lorelli et al, 2002[11]	53.0	25.0	5	27.4	18.7	13	14	1.19 (0.07, 2.31)	0.04	Amersham, UK
Nakamura et al, 2009[12]	38.5	31.4	4	28.2	43.3	18	17	0.24 (-0.85, 1.32)	0.67	SRL Inc, Japan
Overall	-	-	37	-	-	90	100	1.42 (0.48, 2.36)	0.003	-

Figure 1: PRISMA Flow Diagram









Quality Assessment

Study quality was assessed in relation to the reporting of the following items within the publications of the included studies: 1) study design and setting and whether the primary outcome measures were related directly to MMP-9 concentrations and the presence of endoleak; 2) definition of the endoleak and control groups; 3) methods of patient recruitment, definition of inclusion and exclusion criteria and baseline characteristics of the patients; 4) assay type, protocol and quality of assay results for measuring MMP-9 concentrations (such as assay precision and interassay coefficient of variation) and 5) adjusting, excluding or matching for confounders. Quality was scored as shown in Supplementary Table 1 and graded as poor, average or good according to Supplementary table 2. Individual study grading is summarized in Supplementary table 3. No studies were excluded due to poor methodological quality. In the studies[8,10,11] that were graded as average, there was a clear study design, good reporting of baseline participant characteristic, primary outcome, assay protocol and variability but poor or no adjustment for confounders. In the studies[9,12] that were graded as poor, there were unclear definitions of baseline participant characteristics, poor reporting of the outcome of interest and no adjustment for confounders.

Supplementary table 1: Quality assessment questions				
Quality category	**Explanatory Questions**	**Response**		
		Yes	No	Unclear
Did the study investigate the main primary outcome?	Does the study investigate MMP-9 levels in patients with and without endoleaks?			
	Is the study defined according to study type (case-control, RCT etc)			
	Is the study setting defined (e.g. hospital based; single/multi-centred)?			
Is the study population sample well-defined?	Is the recruitment of participants conducted in a geographically defined region?			
	Are the methods for recruitment/sampling detailed in the study?			
	Are inclusion and exclusion criteria detailed?			
	Are controls appropriately matched to the cases (i.e. minimum gender and age)?			
	Are the baseline characteristics of patients detailed?			
Did the study define the groups for comparison adequately?	Was the definition of endoleak in patients adequately defined?			
	Was the diagnosis of endoleak in patients adequately defined (type of imaging used)?			
Are the controls defined properly?	Was the negative diagnosis (no endoleak) adequately defined? How was the negative diagnosis defined?			
	Was the time period for diagnosis for no endoleak defined in the study?			
Was MMP-9 level measured by specific	Was the assessment of MMP-9 one of the main study aims?			

defined methods?	Does the study report on plasma or serum MMP-9 (blood medium)?			
	Does the study detail the assay method used for assessment of MMP-9?			
	Does the study detail the protocol used for MMP-9 measurement (incl. blood collection and blood medium used; eg. plasma, serum, whole blood)?			
	Did the study report on the quality of MMP-9 assay results? (e.g. assay precision, interassay coefficient of variation)			
	Did the study report on timeline of measurement of MMP-9 levels?			
Did the study exclude, match or adjust for the following confounders as listed?	Age?			
	Gender?			
	BMI?			
	Hypertension?			
	Smoking status?			
	Diabetes?			
	Presence of or renal function?			
	Presence of CVD?			
	COPD?			
	Use of other medications?			
	Previous vascular surgery?			
	Previous endoluminal access?			
Does this study show any publication biases?	Does the study report findings in relation to the original aims?			

	Does the study report findings in the context of the existing literature? (Study rationale)			

Supplementary table 2: grading system for studies			
Quality category	**Poor**	**Average**	**Good**
Study design	Less than 1 criteria met	1-2 criteria met	3 of 3 criteria met
Participant characteristics	Less than 2 criteria met	2-3 criteria met	At least 4 of the 5 criteria met
Endoleak cases and definition	Less than 1 criteria met	1 of 2 criteria met	2 of 2 criteria met
Control cases definition	Less than 1 criteria met	1 of 2 criteria met	2 of 2 criteria met
MMP-9 measurement	Less than 3 criteria met	3 -4 criteria met	At least 5 of the 6 criteria met
Confounders	Less than 1 criteria met	2 of 12 criteria met (age and gender)	At least 3 of 12 criteria met
Publication bias	Less than 1 criteria met	1 of 2 criteria met	2 of 2 criteria met
Total	Less than 16	16-24	More than 24

Supplementary table 3: Quality assessment table (consensus)					
Quality category	Monaco *et al.* (2007)[8]	Hellenthal *et al.* (2012)[9]	Sangiorgi *et al.* (2001)[10]	Lorelli *et al.* (2002)[11]	Nakamura *et al.* (2009)[12]
Study design	Good (3)	Average (2)	Average (2)	Average (2)	Average (2)
Participant characteristics	Good (4)	Poor (1)	Good (4)	Average (3)	Good (4)
Endoleak definition	Good (2)	Average (1)	Good (2)	Good (2)	Good (2)
Control-cases definition	Average (1)	Average (1)	Good (2)	Good (2)	Poor (0)
MMP-9 measurement	Good (5)	Average (3)	Good (6)	Good (6)	Good (5)
Confounders	Poor (0)	Average (2)	Poor (0)	Poor (0)	Poor (0)
Publication bias	Good (2)	Good (2)	Good (2)	Good (2)	Good (2)
Total score	17	12	18	17	15
Overall grade	Average	Poor	Average	Average	Poor

Supplementary table 4: Sensitivity analysis using one study removal approach		
Study Removed	Standardized mean difference	P value
None	1.42 (0.48,2.36)	0.003
Monaco[8]	1.44 (0.27,2.62)	0.02
Hellenthal[9]	0.99 (0.46,1.52)	0.0002
Sangiorgi[10]	1.47 (0.23,2.71)	0.02
Lorelli[11]	1.47 (0.29,2.65)	0.01
Nakamura[12]	1.71 (0.76,2.66)	0.0004